Lecture 18 Experimental Design

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In this lecture I'm going to talk to you about how to design an experiment. And when I talk about experiment design here, I'm talking about experiments where you manipulate one variable in order to observe an effect on a different variable. I'm not going to be talking about observational studies where you go into the field and you count organisms or you measure things in the field. This will be highly controlled manipulations. In the image here, you can see the Jena experiment that we've looked at in previous lectures. You can see that the piece of grassland here in Germany is divided into plots, and each plot has a different community. This has enabled the researchers to make comparisons between different communities for soil nutrients and insect life and plant species diversity and all sorts of different things, just by changing these communities.

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By the end of this lecture, we should be able to design a controlled experiment. And I'm going to emphasize that you really need to be able to explain your choices that you make. Everything that you include in your design has to be carefully considered and well justified.

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An experiment in this context is where you manipulate one or more variables and then you will measure that effect on another variable. The manipulated variables are independent variables. These are the cause of the effect, and these are the things that you change. It could be temperature, could be moisture, could be light, could be other species.

The dependent variable is the variable that you are looking at. You’re looking to see a change in the dependent variable. You might look at the soil microbial community, and if you've changed the moisture, you might be looking for a change in species richness of the microbial community.

My example here is the coral reef that you see in the picture. One example is: will an increase in temperature from climate change affect coral populations? Remember we talked about how sensitive coral is in the last lecture. Your independent variable is temperature that is the thing that you are changing, and the dependent variable is the current population and that is the thing that is being changed.

There are other names for independent variables that if you do reading around experimental design you my come across independent variable, also known as explanatory variables or predictor variables.

Dependent variables, so the things that are changing, are also called response variables or outcome variables. That might actually be easier to explain. When we're graphing our data, our findings from our experiment, the independent variable goes on the x axis at the bottom of the graph and the dependent variable goes on the Y axis, which goes up the left hand side.

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When we get started, we need to frame our research question and it is crucial to understand where the knowledge gaps are in the field of your study. You don't want to be doing things that everybody's known about for a century, your research is hopefully novel new exciting innovative and for this we need to know where the gaps are and what needs answering. And so you'll need to do lots of reading around the subjects to find the gaps.

Then you can start to frame your question. Examples of research questions are, how will increased temperature affect fish populations on a coral reef, or how do plants change soil nutrients? Your research question is exploratory.

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Having come up with your question you need to then come up with a list of variables that are important. In many instances, there are a lot of variables that might not affect your response variable (dependent variable). You need to design an experiment where you are reasonably confident that your independent variable will actually have an effect on the dependent variable.

At the beginning then, you will list dependent and independent variables and you need to make sure that there is a potential link between them. That one acting on the other is strongly rooted in theory.

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You need to watch out for confounding variables. Confounding variables are likely to have impacts on your dependent variable. But you are not interested in this. It is inevitable that this is going to happen anyway. You need to make sure that they don't mess up the clear relationship that you're looking for between your independent variable and your dependent variable. Or, you just have to include it, and there's various ways to do this.

For example, if you're interested in plants and whether they will change their growth under climate change, as it gets warmer. You also need to think about water because as it gets warmer, the water in the soil will evaporate. This is likely to be a problem because when you measure growth of plants you then wouldn't know if it's the temperature or the water loss that is causing the effect. You need to control the water. Otherwise, the people that look at your results can say, well, we don't know if it is the temperature or if it's the water.

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Then we write our hypotheses. The hypothesis is a statement that we can test. Hypothesis testing is a formal procedure for investigating our ideas about the world. We make specific predictions. This again arises from background literature. A hypothesis is different to a research question. It is a falsifiable statement, which means that when you've completed your experiment and you've looked at your data, you can say yes. The hypothesis is true, or no, it is false and thus you reject the hypothesis.

You create a null hypothesis. And this is a statement that says there is no effect of the independent variable on the dependent variable. For example here, your null hypothesis is Myanmar has no bat species.

Your alternate hypothesis notated as he would be the opposite. Myanmar does have bat species. Then you would set out to test whether you must accept the null hypothesis or the alternate hypothesis.

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When you're designing the experiment, you need to think about where you will do it and how you will do it- how you will carry it out to get your results. And there are obviously many instances where it has to be out in the field out in a natural environment. When we say in the field, we mean just outdoors, the natural environment. It does not mean literally a field. For example, if you're looking at tigers or rainforest species or something like that, it would have to be in a field.

Other things like microbial activity or plant species interactions can be done in the laboratory or the greenhouse. It’s just a case of thinking about what is most appropriate to your scale- to the length of time you have, what is best in order to measure your effect. It can be quite difficult to manipulate things in the field. There are many ways to do it.

All of these approaches have their advantages and the disadvantages. The field has more realism, but there can be a lot of confounding variables. There could be other animals that come in and destroy your equipment or you get wind and rain and strange weather effects and all sorts of possible confounding variables in the field. The greenhouse is less real. But it is more easily controlled so you can control light temperature, moisture and so on in the greenhouse. This is useful; the greenhouse is very useful for plant experiments. In the lab is very tightly controlled. It's great for small mechanistic studies where you want to really understand how something affects something else, very good for microbial studies or soil or seeds. But there is an argument that your results may not translate into real life.

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So once you've decided on your location, you will then decide on your experimental treatments. What change are you interested in? And so as mentioned this could be water availability. It could be temperature, nutrients or other species. Lots of exciting experiments on species interactions. How can you make these manipulations? Some things might be quite difficult to do. pH in the soil would be quite difficult. Whereas temperature, water- these things are fairly simple to change.

You need to consider as well, how big a change this will be. Will you stay within the limits of realism?

And how many changes will you make? For drought, you could do a very severe drought and then a less severe drought. You will need to make those kinds of decisions and you will need to justify it, of course. Why have you chosen to do it the way you've done it?

With our climate change lecture, we said that a four degrees centigrade increase is the worst case for the end of the century. And you could design an experiment where you increase temperature one degree, two, three and four and look at the change in your dependent variable at each of these temperatures or you could look for a limit. So you could say, well, I'm going to look for the point where everything stops, maybe, like enzyme activity. You would just push the temperature really high. You could go up to 20 degrees, for example, higher than what we're expecting and look for the point where you might get a big change, or a big death or, you know, end of activity and then you would find that threshold.

And then you would consider whether your variables are categorical or continuous so categorical is things like blue green. Yes. No. Up, down, you know, categories. Continuous variables are things like pH or height or length, things that the numbers don't have actual categories.

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It is absolutely crucial when you design an experiment like this that you have an unmanipulated control. And this is so that you can see the effect of your treatment. Otherwise, you have no way of knowing that the effect you've seen is not just time. This is particularly critical in things like if you're looking at a plant community, because they of course will change over the growing season. Let's say between May and September these plants are growing, they will flower, they will seed and they will start to die. You need to be able to control for this. Otherwise, the effects of your treatment, we might think, oh, well, how do we know it's different from the normal seasonal progression of the plants? You would have a control group to account for that normal seasonal progression and then you can measure the effect of your treatment compared with that. For example, if you have a drought treatment, you will need a treatment that has the normal range as well. And then you would compare the effect of the drought with your normal rainfall and if you have chosen to do your experiment in the lab or in the greenhouse, you will need to create a normal rainfall or a normal set of conditions.

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If you have two or more independent variables- two or more treatments, a factorial design will look at every combination of these treatments. It is most simple to measure and simple to understand and interpret if you have looked at every combination of treatments, every possibility.

A factor is your independent variable and it will be categorical so things like drought, warming and so on. And each factor than is divided into levels. You would have a drought factor with the levels drought and well-watered.

I've just put a small schematic at the bottom here, and you can see every combination of the four treatments.

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We must consider the study size. In an experiment “n” is the number of samples you have. Maybe the number of plant pots or the number of animals and ideally in statistics you want at least n = 30. And that is kind of the rule of thumb in order to observe an effect if an effect is there to be observed. But this might not always be possible. It might be very expensive. It might be very time consuming to do that number. And so it's useful to do a pilot study like an initial study just to see how many samples you can process in a day or how many animals you could trap in a night and process.

When you're planning, you need to make sure that you've got as big a study size as possible, but that you can still achieve every step within a reasonable time frame. If you were doing a soil experiment and it takes an hour to process 10 samples, then you could reasonably do it in a day, but any more than 80 starts to become not feasible. You need to consider this for each of your experiments.

But of course, there needs to be enough samples to represent the range of outcomes. You do want as many as possible. And it does increase statistical power. That means that if an effect has occurred from your treatments you will be able to detect it. And if there are outliers, if you have a strange case or just a funny number, it means that you can say, okay, that's not right. And then you can remove it.

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And to do this we need to replicate our experiments. Each of your treatments so drought or warming, it must be replicated. You must have replicates that are identical. And then you will have a group of identical replicates that you measure.

If you only have one of your treatments, you might get a graph like this. We have independent variable at the bottom categories 1,2,3,4. And here it looks like number two is much higher than all the other independent variables. We might be tempted to say that group two is more responsive to the independent variable than one, three, and four. But we only have one instance of each of these variables. There is no replication here. We don't really know.

If you have more replicates you can have more faith in your data. This graph has the same points from the previous graph. But now if you look at group two, you can see that that high value that looked like it was going to mean that it was very different, is actually an outlier. You need as many replicates as you can in order to be able to detect a difference and also detect problem numbers and problem samples. Now it looks like group four is the highest.

As a rule of thumb, we say at least four replicates per treatment in order to have faith in your data.

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Your experimental subjects must be randomized as well. Let’s say you have 100 pots in your greenhouse and you have drought and non-drought (control). You would have a list from 1 to 100 and you would randomly assign each pot to either drought or non-drought. And then you can either put them into blocks. You might have a block of 10 on one side of the greenhouse and then another block and then another block. And each block will contain representatives of each treatment or you might just randomly intersperse the pots just all around the greenhouse. But randomization is extremely important. Because what can happen is that if you have everything in order, things might change over the course of your measurements and this might start to become apparent when you come to look at the data. You might get tired and less careful or the light might change over time or, you know, something might change. And you need to have everything randomized so that it doesn't affect one treatment more than the others.

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Block designs as well are very useful for statistics. You would put one representative of each treatment in a block. And this is really useful if there are things like things that are going on in your site or in your greenhouse that you can’t do anything about. A slope in a field could be a good example, because if you were looking at soil nutrients at the top of the slope, it tends to be drier than the bottom of the slope, the bottom of the slope will have more water because water travels down. And what you would do is that would become a confounding factor this moisture change. But if you did a block at the bottom of the slope and a block halfway up and a block at the top and you had each treatment represented in each block. Then that would be a good way to deal with that factor because you know that soil nutrients are linked closely with soil moisture.

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Then you take your measurements, you've set up your experiments. You run it for as long as you think is appropriate and then you take your measurements. Now it's quite useful to do a measurement of your samples before the treatments. You might take a small sample of each of your soils before you start to drought the plant. When you measure your samples each sample does need to be the same as the others if possible. You must do everything exactly the same. And it does help usually if you can take the measurements within a short time frame if possible, but if that's not possible, then this is where block designs could be useful as well because you could do one block one day and one block the next. That’s quite a useful thing to do as well.

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One thing that is often incorporated into studies is doing repeated measures on the same samples and looking to see if those samples change. This is very powerful and a very good way of determining the effect of your treatment and especially if you think that there is a season or effect. So, like, with the plants it is quite useful to test repeatedly maybe once a month over the course of the growing season. And you remember the lynx and snowshoe hare example. There was a question of how many times should Krebs have taken the measurement. And of course, we know that the lynx and the snowshoe hare has a cycle of eight to 11 years so to measure them again and again they captured that very nice relationship between the two animals.

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Then once your treatments are done your data is collected you analyse and I will talk about this more next week.

And if nothing has happened, despite all of your efforts and all of your wonderful experimental design, nothing has happened, you accept your null hypothesis. This is disappointing. Or maybe not. Maybe you don't want an effect.

Then there's a big data preparation stage where you enter into the computer. You clean it of strange values wrong values, check it through and then you save it. The data are sacred once they're put in. You must never ever touch them, change them, nothing

You also store the metadata, and metadata is information about how and when the samples were taken so that other people can also run the experiment if they want to, so they can copy your results and also if anything strange comes up later. You have a paper trail in order to go back and check. Then, next week, we're going to talk about simple descriptive data, descriptive statistics and visualizations and to look at what the data shows, and in our final lecture, we're going to be doing inferential statistics. We'll be testing hypotheses and models.

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I'm going to talk to you now about an actual example. This is some work of mine and the Wessex BESS experiment was based in the southwest of England on Salisbury Plain, which is this large area on the map here. And Wiltshire is of interest because it has a very extensive area of calcareous or chalk grassland, and you can see it's in the green here. And all the yellow is where they're trying to restore this grassland, and our project Wessex BESS is in this region, BESS stands for biodiversity and ecosystem services and sustainability.

And we were looking at how to restore ecosystem function and ecosystem services in this chalk grassland, and chalk is particularly precious. It's very poor soil and it and it hosts the most diverse grassland. The highest diversity of any grassland in Europe, it is extremely valuable. And the reason is so diverse, is because it's very nutrient poor so everything grows very slowly and nothing has the opportunity to dominate.

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Our research questions are what ecosystem functions are delivered in mature grassland and we were particularly interested in communities. If you have a change in plant community, do you get a change in the functions delivered?

And then we were also interested in the effects of climate change and in the UK drought is the most pressing question.

We wanted to know that if you have different plant communities that deliver different functions, then when they get droughted would this change ecosystem functions differently? Does each group respond differently?

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Our hypotheses; and this is kind of knitting together everything that we've looked at over the last few weeks. We hypothesized that plant species with functional traits- you remember the trait characteristics? Exploitative resources, these ones that are looking for lots of nutrients in the soil and take it up very rapidly. We hypothesized that this would mean that there are very increased ecosystem function rates if we have plant species that have very exploitative traits and make use of all of their surroundings. Do we have faster decomposition and faster nutrient cycling? And from the literature, we found that exploitative functional traits are not equipped to deal with abiotic stress. Basically plants make a choice. They only have a certain amount of resources and so they must choose to either put it into strong structural carbohydrates, so that they are around for a long time and therefore grow more slowly, or they will put it into making fast growing cheap easily broken down roots and so on, that will grab lots of nutrients that are quite poorly made so it's just about speed and grabbing any resources, they can. And so we thought that these exploitative species, when you give them a drought when climate change happens, then they will be badly off, they will be badly affected and this will be seen in the ecosystem functions that there'll be a big effect.

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My design for my experiment: I created three functional groups and these are all thought to have different effects on the environment.

And with their rooting structures we can say that group one and group two would have lower ecosystem function rates, but they will be more resistant to drought, whereas group three has this very fibrous root system, and it's all in the top of the soil and everything will move very fast. Lots of decomposition lots of nutrient cycling. But when we drought, this system will show a very big drop in ecosystem functions.

We created seven combinations: all three of these groups on their own. 123 and then pairs. So, this is planting them into the field. We had them in pairs. 1&2, 1&3, 2&3, and then all three groups together (123). And we replicated this six times. Seven times six is 42 plots.

But there was a slope in the field that we were planning to do the experiment. And so we had to create a block design. We would have seven combinations at each level of the slope. I will show you this here.

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This is the experiment. And we've got some photographs of it at the bottom. And what you see here, this is my design of the experiments at the top we have the top of the slope, at the bottom we have the bottom of the slope. And each horizontal row is one block. And you can see here that each plot

has one of the combinations of the plant functional groups, F, G stands for functional group.

We have block one at the top and block two the next one down all the way down to block six and each one has all seven combinations randomly located.

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Now within one plot, each one was eight meters by eight meters and we split the plot into zones and this is very important because we carried out repeated measures. The experiment around for three years and we measured three times each year and we would do vegetation surveys in these fixed plots in the top left. We also had an experimental zone on the right and that was where we did our gas measures so greenhouse gases. And there was a fixed ring. It was always the same. And so you can make comparisons over time. Each of the plots has this exact same layout and the experimental zone was where we collected soil samples three times per year.

Now RC and UC and D. These are our climate change treatments and they are shelters that keep the rain out. RC is the roof control, remember how I talked about the importance of a control. And what we have here because I expect that the shelter will give microclimate effects. Under the shelter, you might have it very humid and more warm.

And so the D for drought. that has no rain allowed in for six weeks. We have RC, which is roofed control, which means that we have the roof and but it is a control and so it has holes in to allow the rain fall through. We have accounted for the microclimate effects and that we've also allowed the water through. So, that is how we have attempted to match the conditions and control for these confounding effects in the field and you see is Unroofed Control. That is a small plot that had no roof or rain shelter.

The other thing I want you to notice is that all of these experimental sections and the roofs are at least one meter from the edge of the plot. And this is another case of edge effects. And to account for edge effects we move all of our experimental equipment and so on closer into the centre of the plot so that we don't have this problem with edge effects.

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I will share the paper with you that I published on this. This is a picture of the experiment and in the foreground. This shelter, here you can just see the holes that have been drilled in through the plastic to allow the rainfall, just to come through and then there's a gap. And then there's the drought and control treatment. And you can also see the gray collar underneath the shelter here, and that is to measure greenhouse gases. That is fixed. So we would come, three times per year to measure greenhouse gases inside that ring.

And so we created this high emissions scenario. Six weeks 100% drought.

We had some very exciting results from this we have published in Ecology some work on multifunctionality which we talked about and ecosystem functions and I can share this paper with you.

This is just an example of a highly controlled manipulated study.

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In summary, experiments must be carefully planned.

Everything you decide you must be able to justify, why you've made this choice and think about your statistics and your analysis right at the beginning.

It will save you a lot of pain and sadness at the end if you already know what statistics you will use and the way that you will present and visualize your data.

Reading

https://www.scribbr.com/methodology/experimental-design/

https://www.itl.nist.gov/div898/handbook/pri/section3/pri3.htm sections 1-3 only

https://explorable.com/experimental-research

<https://conjointly.com/kb/research-design/>

Fry et al. (2018) Ecology

Discussion topics

1. Is climate change a threat to ecosystems in Myanmar?
2. Which ecosystems do you think are most affected?
3. Are there any university initiatives to reduce climate change?